

5     **WHAT IS CLAIMED IS:**

1.     A native, authentic, enzymatically active  
NTPase/RNA helicase protein produced by a process  
comprising the steps of:

10             a)     expressing an NTPase/RNA helicase  
encoding nucleic acid of hepatitis C  
virus in a eukaryotic expression system  
such that a complete, authentic and  
native NTPase/RNA helicase protein is  
synthesized, said authentic and native  
15     NTPase/RNA helicase protein comprising  
amino acids 1027 -1657;

20             b)     extracting NTPase/RNA helicase protein  
from said eukaryotic expression system  
in an enzymatically active form of said  
protein; and

              c)     purifying said NTPase/RNA helicase  
protein such that the enzymatically  
active form of said protein is  
maintained.

25     2.     The protein produced according to claim 1,  
said nucleic acid of hepatitis C virus in step a)  
corresponding to a human hepatitis C virus nucleic  
acid.

30     3.     The protein produced according to claim 1,  
said nucleic acid of hepatitis C virus in step a) being  
derived from a genotype of the human hepatitis C virus  
nucleic acid.

4.     The protein produced according to claim 1,  
wherein said nucleic acid of hepatitis C virus in step

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5 a) is a variant of the human hepatitis C virus.

5. The protein produced according to claim 1,  
said nucleic acid of hepatitis C virus in step a)  
encoding a complete NS3 coding region.

10 6. The protein produced according to claim 1,  
said nucleic acid of hepatitis C virus in step a)  
encoding a complete NS3 through NS5B coding region  
comprising amino acid residues from 1027 to 3011 of  
hepatitis C virus genome.

15 7. The protein produced according to claim 1,  
wherein said expression system is a recombinant  
baculovirus-insect cell expression system.

20 8. The protein produced according to claim 1,  
wherein the extracted protein is purified by  
immunoaffinity chromatography using antibodies specific  
for hepatitis C virus proteins.

9. The protein produced according to claim 1,  
having basal NTPase activity in the range of 0-200 min<sup>-1</sup>  
and RNA helicase activity greater than 0.001 min<sup>-1</sup>.

25 10. The protein produced according to claim 1,  
having basal NTPase activity less than 150 min<sup>-1</sup> and  
RNA helicase activity greater than 0.005 min<sup>-1</sup>.

11. A process for preparing native, authentic,  
enzymatically active NTPase/RNA helicase protein  
comprising the steps of:

30 a) expressing an NTPase/RNA helicase  
encoding nucleic acid of hepatitis virus  
in a eukaryotic expression system such

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5                   that a complete, authentic and native  
NTPase/RNA  
                  helicase protein is synthesized, said  
                  authentic and native NTPase/RNA helicase  
                  protein comprising amino acids 1027-  
10                  1657;

b)       extracting NTPase/RNA helicase protein  
          from said eukaryotic expression system  
          in an enzymatically active form of said  
          protein; and

15       c)       purifying said NTPase/RNA helicase  
          protein such that the enzymatically  
          active form of said protein is  
          maintained.

12.   The process according to claim 11, said nucleic  
20   acid of hepatitis C virus in step a) corresponding to a  
complete NS3 coding region.

13.   The process according to claim 11, said nucleic  
acid of hepatitis C virus in step a) corresponding to a  
complete NS3 through NS5B coding region.

25   14.   A native, authentic, enzymatically active  
NTPase/RNA helicase protein product produced by a  
process comprising the steps of:

a)       expressing a nucleic acid sequence in an  
          expression system, thereby producing an  
30       enzymatically active, native, full  
length hepatitis C virus NTPase/RNA  
helicase protein that comprises the  
amino acid residues having sequence  
numbers from 1027 to and including 1657,  
35       wherein said expression system is a  
eukaryotic expression system;

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- 5                   b)     extracting said protein from said  
                    expression system, such that the  
                    extracted protein is in an enzymatically  
                    active form;
- 10                   c)     purifying said extracted protein from  
                    step b) such that the purified protein  
                    is an enzymatically active, native,  
                    full-length hepatitis C virus  
                    NTPase/RNA helicase protein.

15           15.    A method for assaying a compound for anti-viral  
                  activity against hepatitis C virus comprising:

- a)     providing enzymatically active, native,  
                  authentic hepatitis C virus NTPase/helicase protein;
- b)     contacting said protein with a compound  
                  suspected of inhibiting helicase activity; and
- 20                   c)     measuring inhibition of the helicase  
                  activity in said protein by said compound.

                  16.   A method for assessing a compound for  
                  anti-viral activity against a flavivirus, comprising:

- 25                   a)     providing enzymatically active, native,  
                  authentic flavivirus helicase protein;
- b)     contacting said protein with a compound  
                  suspected of inhibiting helicase activity; and
- 30                   c)     measuring inhibition of the helicase  
                  activity in said protein by said compound.

                  17.   A method as claimed in claim 15, wherein  
                  multiple compounds are assayed simultaneously.

                  18.   A method for assaying a compound for  
                  anti-viral activity against hepatitis C virus  
35                   comprising;

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- 5           a) providing an enzymatically active, hepatitis C virus NTPase/RNA helicase protein;
- b) providing a partially duplex substrate in which both strands are RNA and at least two nucleotides at the 3' end of at least one RNA strand are not  
10 involved in base pairing and at least one of said RNA strands is detectably labeled;
- c) exposing said NTPase/RNA helicase protein to said partially duplex RNA substrate in the presence of a putative antiviral compound;
- 15           d) capturing any detectably labeled single stranded release strand product of the interaction between said RNA helicase protein and said substrate with a capture system comprising a specific binding pair, one member of said specific binding pair being  
20 conjugated with an oligonucleotide having a nucleotide sequence complementary to said detectably labeled release strand and the other member of said specific binding pair being affixed to a solid support; and
- e) quantitating detectable label present in  
25 said release strand, as a measure of the anti-viral activity of said compound.

19. A method according to claim 18, wherein the other member of said specific binding pair is  
30 affixed to a mobile solid support.

20. A method according to claim 18 in which said oligonucleotide of said capture system is DNA.

21. A method according to claim 20 in which  
35 said capture system comprises said oligonucleotide conjugated with biotin and agarose beads coated with streptavidin or a derivative thereof.

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- 5            22. A method as claimed in claim 18, wherein multiple compounds are assayed simultaneously.

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